Appearance of sperm activation factors in the ovary of the sea urchin *Hemicentrotus pulcherrimus* with maturation[1]

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Summary. Ovaries of the sea urchin *Hemicentrotus pulcherrimus* were extracted with 70% ethanol. The extracts were polarographically assayed for respiration-stimulating activity toward homologous spermatozoa. The data of the bioassay and light microscopic observation on thin sections of the ovaries showed detectable activity only in mature ovaries.

The respiration and motility of sea urchin spermatozoa are markedly reduced at low pH-values[4,5]. The pH-value within the jelly coat of a sea urchin egg seems to be lower than that of normal sea water[5,6]. Thus, the egg jelly is considered to be provided with a system for maintaining the respiration and motility of spermatozoa at an initial high level in normal sea water during passage through the acidic environment of the jelly coat to the egg surface.

Recently, peptides which stimulate the reduced respiration of sea urchin spermatozoa were purified from the egg jelly coat of several species of sea urchins[7,8] and the structure of 2 peptides of *H. pulcherrimus* was established[8]. Moreover, there is evidence suggesting that the transport of Na^+ and/or H^+ across the sperm cell membrane is of prime importance in stimulating sperm respiration and motility by peptides[9].

However, many investigators[4-8] have been interested in sperm activation factors or peptides in the egg after spawning. Until now, there has been no report dealing with the existence of the factors or peptides in the egg before spawning. We think that it is important to investigate whether or not the factors or peptides are present in the ovary during the whole process of ovary maturation, since it is essential for the investigation of peptide biosynthesis. In this paper, we report the relationship between the appearance of respiration-stimulating factors in the ovary and ovary maturation.

Materials and methods. Sea urchins, *H. pulcherrimus*, were collected once a month from October, 1980 to September, 1981 from the sea coast near Misaki Marine Biological Station. The sex of *H. pulcherrimus* was easily identified by the color of the tube feet; the tube feet of the female are

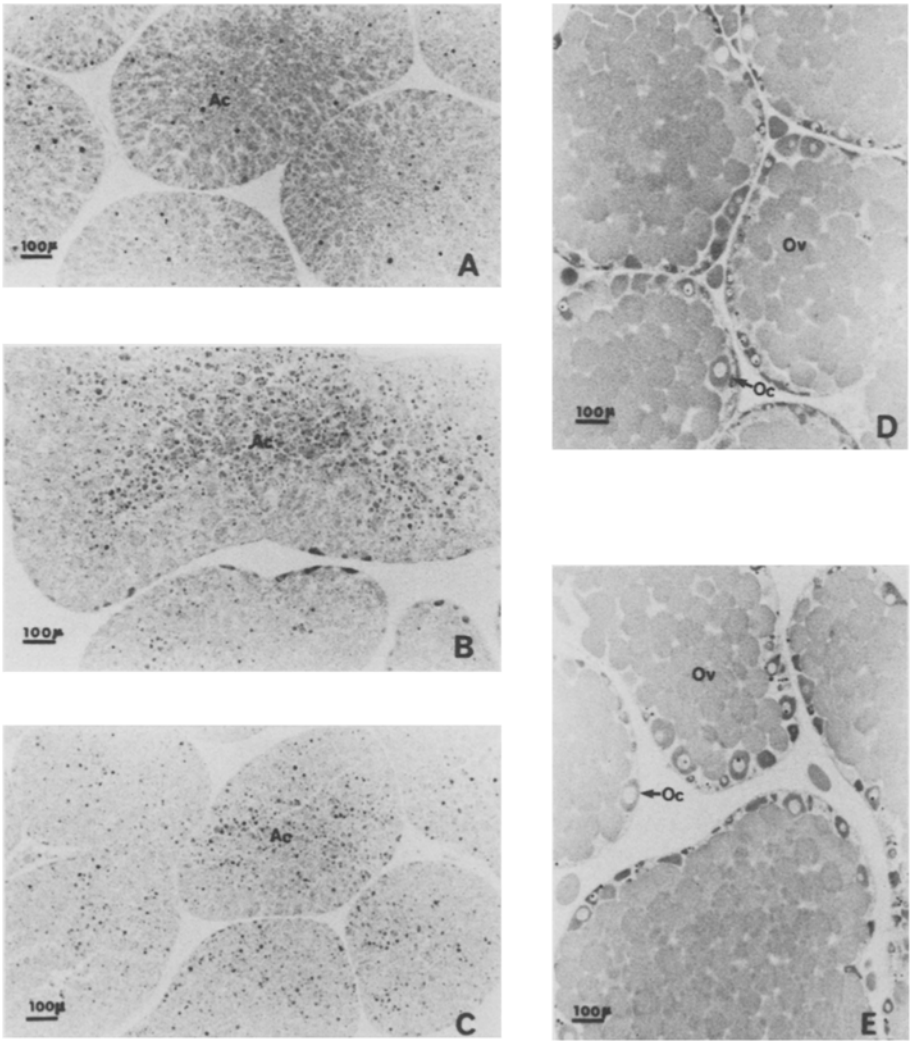

Figure 1. Thin sections of paraffin-embedded ovaries of *H. pulcherrimus* collectd in October (A), November (B), December (C), January (D) and February (E). Ac represents the area of accessory cells; Oc, oocyte; Ov, ovum.

dark yellow and those of the male white. The ovary was isolated and cut into 2 pieces. One piece (about 1 g) was fixed with Bouin's fluid for the histological examination. The other piece (about 4 g) was homogenized in 40 ml of 70% ethanol with a glass-fitted homogenizer and then centrifuged at 10000×g for 30 min at 4 °C. The precipitate was lyophilized and weighed. The weight of the precipitate was considered as the dry weight of the ovary. The supernatant was dried under reduced pressure below 50 °C and the residue was dissolved in 2 ml of distilled water. An equal volume of chloroform was then added to remove the lipids. The aqueous part was withdrawn and lyophilized. The residue was kept in a freezer at −80 °C until the activity was examined. The respiration-stimulating activity of the sample for the *H. pulcherrimus* spermatozoa was assayed by polarographical measurement of oxygen consumption in a sperm suspension as reported elsewhere[5]. The activity of each sample was expressed by dividing the rate at half-maximal stimulation by the dry weight of the ovary. For histological examination, a fixed piece of an ovary was dehydrated with an ethanol series and embedded in paraffin. Sections 8 µm thick were cut and stained with Mayer's hematoxylin and eosine.

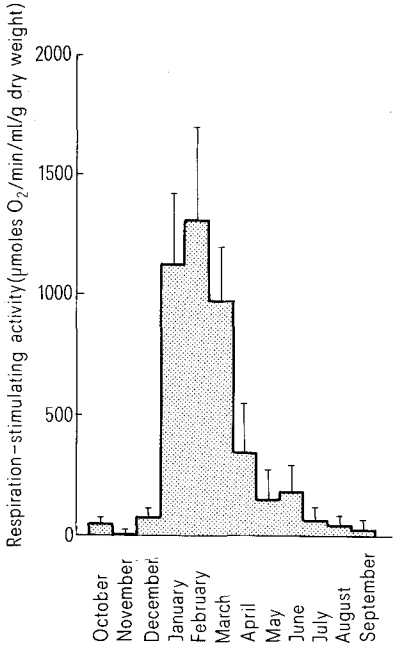

Figure 2. Annual cycle of respiration-stimulating activity in extracts from ovaries of *H. pulcherrimus*. 5 specimens were collected on October 10, November 21, December 11, January 8, February 7, March 10, April 12, May 10, June 9, July 11, August 8 and September 9, respectively. Thin sections of the specimens from October to February were examined under a microscope.

Results and discussion. Figure 1 shows cross sections of *H. pulcherrimus* ovaries. From October to December, the ovaries were filled with accessory cells containing globules (fig. 1, A–C). The reproductive season began at the end of January in 1981. In January and February, mature eggs occupied all the space within the ovary (fig. 1, D and E). These morphological changes were essentially the same as those reported by Masuda and Dan[10]. They indicated that ovaries from April to November remained at the same morphological stage. Our interest was in whether these ovaries at different developmental stages had respiration-stimulating activity towards spermatozoa and if not, when this activity appears in the ovary. As shown in figure 2, extracts prepared from ovaries from January to March showed high respiration-stimulating activity, but extracts from ovaries between April and December did not show as much activity. Furthermore, extracts prepared from testes showed no detectable respiration-stimulating activity. These results indicate that the appearance of the respiration-stimulating activity parallels the maturation of the ovary and the peptide is present before the egg is spawned, since no ovaries spawned naturally or artificially in January. Therefore, it is concluded that the peptide is not synthesized by the egg after spawning. At present time, we do not know which cells are responsible for the peptide synthesis, nor how the peptide is transported into the egg jelly coat, but we think that our results will be useful for the study of the biosynthesis of the peptide.

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Effects of monosodium glutamate on circulating concentrations of luteinizing hormone and growth hormone in young growing domestic fowl *(Gallus domesticus)*[1]

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Summary. The administration of monosodium glutamate (MSG) at a dose of 4 g/kg in 5-day-old fowl or 5 daily injections of MSG (total dose 20 g/kg) in 1- to 5-day-old fowl did not affect body growth in either male or female domestic fowl. Neither MSG treatment schedule affected either testis weight or the circulating concentration of luteinizing hormone (LH). A small, but significant decrease in the plasma concentration of growth hormone (GH) was observed in female chicks which had received daily MSG injections.

Postnatal administration of high doses (4 g/kg) of monosodium glutamate (MSG) to laboratory rodents induces lesions in the hypothalamus[2]. In particular, there appears to be destruction of neurons in the arcuate nucleus[3]. This is accompanied by decreased concentrations of dopamine and an enzyme of cholinergic synapses (choline acetyl trans-